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13. ABSTRACT (<i>Maximum 200 Words</i>) Parkinson's disease (PD) is caused by deterioration of the dopamine (DA) nigrostriatal system. Loss of DA can be induced specifically by neurotoxic lesion of DA neurotransmitter producing somata in the substantia nigra, or through lesioning DA terminals in the striatum with subsequent degeneration in substantia nigra. Characterization of an animal model of PD after bilateral intrastriatal infusions of the neurotoxin 6-hydroxydopamine has been initiated in the first period of this project. Different doses of the neurotoxin in multiple striatal infusion sites were tested, and treated animals were followed for two months. Behavioral assessments of the motor impairments produced by the neurotoxin treatment were evaluated using the Montoya staircase apparatus for skilled reaching to evaluate dexterity and motor sequencing ability in the forepaws. It was determined that this apparatus also assessed posture and balance abilities. This reduced the number of procedures needed to examine behavioral deficits in neurotoxin treated animals. Neurochemical analysis of residual striatal DA four weeks following neurotoxin infusion is being determined using HPLC of the DA and its metabolites. Morphological examination of the extent of the losses in striatal DA terminals and DA receptor subtypes will be established in neurotoxin-lesioned brains that are presently stored and awaiting analysis.					
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FOREWORD

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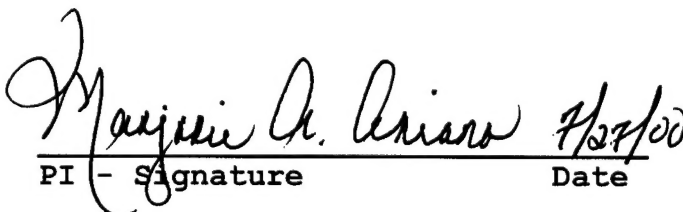

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Introduction:

This project is evaluating whether substantia nigra DA neurons may be damaged initially without showing profound loss of function assessed using various behavioral, biochemical and anatomical measurements. Residual DA neurons in the substantia nigra, and their terminals in the striatum may work more efficiently to overcome deficits produced by partial, bilateral destruction of the nigrostriatal pathway. The classic motor alterations associated with PD in patients include bradykinesia, spasticity, gait disturbances, postural abnormalities and tremor at rest [1]. We will assess behavioral anomalies in rats following partial, bilateral DA loss using the Montoya staircase apparatus. This behavioral task evaluates skilled forepaw reaching [2], motor sequencing, posture and balance. The integrity of the nigrostriatal system is determined using biochemical measurement of DA and its metabolites [3,4]. Striatal DA receptors may show shifts in expression following partial DA denervation, as they do in other unilateral animal models of PD [5-7]. We predict that a subsequent exposure to another stressful event following partial bilateral DA lesions will provoke further changes in the DA system. This will lead to significant changes in striatal DA receptor expression patterns, further behavioral abnormalities, and changes in DA and mitochondrial functional indices in the substantia nigra.

Body:

Preamble: Seven tasks were identified for study in the first year of the project. Our expectations when we submitted the proposal were quite ambitious however, and some aspects of the research plan have not been attempted (details provided below). We did not receive moneys to hire personnel until mid September 1999, at which point the PI had undertaken a sabbatical leave at UCLA. Nonetheless, we made good progress on the behavioral experiments that were our first focus and are now well positioned to carry out the remaining task proposed for Year 01 and 02 in the ensuing funding period. Our decision to develop the bilateral PD model using striatal infusions first was predicated on the ease of identifying this large nucleus stereotaxically and the higher probability of obtaining a 50% loss of DA through this approach. However, we did not anticipate that the rats would be affected so severely by lesions to this site, needing supplemental dietary aid and husbandry to thrive. While this situation was temporary and was overcome by approximately the second week after the lesion, it did change the scope and timing of subsequent experiments. Each of the seven tasks is listed, followed by the work completed to date in the subsequent narrative.

Task 1: *Commence bilateral striatal 6-OHDA and substantia nigra malonate infusions into young adult, male rats using various infusion doses of the toxins to establish the concentration necessary to achieve 50% loss of striatal DA.* Neurotoxin infusions were restricted to one compound, 6-OHDA, since the animals need dietary support and extra attention to flourish following the bilateral insult. The 6-OHDA was infused into the striatum in ascorbate-saline using stereotaxic coordinates to yield four sites per rat (2 per side). Eight animals were subjected to this regimen, using two different doses of 6-OHDA (12.5 µgm/4 µl or 6.25 µgm/4 µl). Another pilot group examined unilateral striatal infusion of the higher dosage of neurotoxin, with subsequent 6-OHDA injection into the contralateral striatum 2 weeks after the initial surgery to assess whether staggered bilateral destruction of striatal DA was better tolerated as an experimental paradigm. The results demonstrated that the lower dose of 6-OHDA would yield an approximately 50% loss of striatal DA. It is notable that all of the animals survived this surgery, but needed dietary supplementation for at least 5 days. The timing of the bilateral neurotoxin infusion was not a factor in the speed of surgical recovery. However, any early behavioral assessments would not be possible due to the post-surgical interventions needed, and bilateral neurotoxin infusions would be given concurrently.

Task 2: *Baseline behavioral assessment using two paradigms, skilled paw reaching and elevated bridge traverse to examine balance, posture and dexterity after the neurotoxic lesion.* We focused on the alterations in manual dexterity because we felt subtle changes in this task would be easier to assess. Additionally we determined that the staircase apparatus also would discriminate changes in balance and posture, thus it might substitute for an alternative behavioral paradigm to examine these motor components. The Montoya staircase apparatus [2,8] tested skilled forepaw reaching for reward pellets following 12 hours of food deprivation. Training included as many as 16 sessions of 15 minutes duration each. Rats needed “shaping” to acquire this activity using baits of sugared cereal (Fruit Loops™ were ideal) on the first 4 stairwells. As rats learned the task we changed to 45 mg Purina reward pellets. Since there are six wells on each side, loaded with two reward pellets, a “perfect” score in the test session would be 24 (see Graph I). None of the rats achieved a perfect score in the test sessions. A total of 8 rats were examined in this behavioral paradigm. This is shown in Graph I in the appended material. All animals showed a learning curve typical for the acquisition of a new motor skill. Successful pellet grasp and consumption (“hit”), attempted grasp (“miss”), and no attempt (“no try”) at the reward were documented for each stair well for each session in which the rat was tested. Records were made of the preferred paw used to reach for the reward to assess “handedness” of the animals. The balance, motor sequencing and duration of the behavior also were recorded. The elevated bridge behavioral paradigm was not examined as components of balance and posture could be determined in the skilled forepaw reaching task.

Task 3: *Bilateral infusions to the striatum (6-OHDA or carrier without neurotoxin), substantia nigra (malonate or only carrier fluid).* These were carried out for 6-OHDA only, on the animals previously evaluated in the skilled forepaw reaching tasks. 6-OHDA was infused into four sites of the striatum (6.25 µgm/4 µl), as determined in Task 1. Animals were given dietary supplementation for 1 week and body weights recorded.

Task 4: *Behaviorally assess the animals produced in Task 3 within 24 hours of the surgery, then extract brains for subsequent biochemical and morphological evaluation.* This short-term behavioral analysis could not be performed due to the status of the neurotoxin treated animals. Thus, rats were evaluated one week after the neurotoxin infusion to ensure recovery from the 6-OHDA infusion. The Montoya staircase apparatus was employed to examine fine motor coordination and postural changes in the lesioned animals. The results were not as consistent in the lesioned animals as the naïve group. This was due to our concern that the animals should not be food deprived because of their tender caloric insufficiency in the first week following the bilateral striatal DA lesions. However, a learning curve was visible, although the animals were not as “motivated” to obtain the reward pellet (Graph II in appended materials). Additional observations were that the animals needed more time to consume the pellet, and as a consequence the 15-minute test session was too short for adequate performance evaluation of the lesioned animals. Postural impairments were detected, consisting of an inability to position the body to reach appropriately for the reward pellet. Motor sequencing also was impaired and chewing was abnormal. These were evaluated by video taped sessions. The animals were killed 24 hours after the last behavioral session and the brains have been frozen.

Task 5: *HPLC assessment of DA and its metabolites on half of the frozen striatum from the animal groups established in Task 3.* Another series of animals were lesioned at the two doses of 6-OHDA and have been sent to our long-time collaborator, Dr. John Elsworth (Yale University) for HPLC analysis. These rats were not behaviorally evaluated.

Task 6: *Morphological evaluation of the remainder of the brains produced in Task 3 will be performed for the DA receptor subtypes, and DA terminals using histofluorescence. The data will be conjoined with results from HPLC and behavioral studies.* The brains from the animals prepared in Task 3 and behaviorally assessed in Task 4 have been frozen and await morphological analysis.

The brains will be processed in August and September 2000 for striatal DA terminal histofluorescence and DA receptor immunochemical expression. We took all of the animals from Task 3 for anatomy analyses because of the small number prepared to date, and the numerous parameters that need to be examined within the striatum in relation to the extent of the DA lesion sites.

Task 7: *Another group of animals will be prepared as in Task 3, and behaviorally tested at 2 weeks and 4 weeks post-lesion. Animals will be terminated within 24-hours of the second behavioral evaluation, brains coded and frozen for subsequent biochemical and morphological examination.* This experiment has not been initiated yet, but will be performed following the morphological analysis of the rat brains on hand.

Discussion: We sought to develop an animal model that more closely mirrors the onset and symptoms of PD in humans using partial, bilateral loss of striatal DA. This was accomplished by intrastratial infusion of the neurotoxin 6-OHDA into the adult rat. To further establish the efficacy of this model, we selected behavioral elements that are altered in idiopathic PD: posture, balance, motor sequencing, and finely coordinated hand skills. We found that all of these elements could be assessed using a single behavioral paradigm, namely the Montoya staircase apparatus typically used to evaluate skilled forepaw reaching. All bilaterally lesioned rats exhibited bradykinesia for up to one week following the infusion of 6-OHDA. Additionally, the rats required dietary supplementation and some needed subcutaneous injections of lactated ringers (10-20 cc BID to QD) to maintain hydration and body weight. The bilaterally lesioned rats lost weight initially but returned to pre-lesion weight within two weeks with supplementation of their diets. However, this outcome makes behavioral assessment at any time point before this recovery impractical, and we will need to alter our experimental paradigms accordingly. Some of these rats have sufficient postural instability that they cannot rear to reach their water bottles, and the animals' husbandry needs careful monitoring. Most bilaterally lesioned rats have dysphagia and need more time to consume the reward pellets used in the skilled forepaw reaching task. Consequently the duration of the test sessions, before and after neurotoxin treatment will need to be lengthened. Other symptoms exhibited by the partial, bilaterally lesioned animals include intermittent spasticity (fisted paws) induced by stress. Gait deviations were apparent immediately after recovery from anesthesia. The initial experiments have provided sound pilot work on development of this alternative animal model of PD with a variety of the hallmark features used to diagnose the human disease.

Summary of Key Accomplishments:

- Bilateral striatal lesions produce transient, but severe dietary consequences, requiring caloric supplementation using specialized liquid diets
- Intrastratial infusion of 6-OHDA (6.25 µgm/4 µl total) in four different striatal sites (2 per side) produce ~50% loss of striatal DA
- 24-hour behavioral assessments cannot be performed due to the status of the rats, immediately following the neurotoxin infusion
- A single behavioral paradigm (Montoya staircase apparatus) is sensitive enough to detect deficits following partial bilateral loss of striatal DA

Conclusions:

This initial period has provided valuable information on the generation and characterization of a bilateral, partial destruction of the nigrostriatal system as a viable animal model of PD. The use of a less dramatic loss of DA to generate this animal model more closely mimics the onset of spontaneous PD in humans than the traditional unilateral DA lesioned rat. The partial, bilaterally DA-lesioned rat provides the opportunity for some recovery within the nigrostriatal system. We had not anticipated the loss of food seeking following a bilateral striatal lesion, and thus our initial statement of work was overly ambitious. Nonetheless all of the animals survived with dietary supplementation, but this did require more husbandry and time to ensure the health of our experimental subjects. We will redesign our experimental protocol to examine different time points following the neurotoxin infusion. We can readily ascertain behavioral abnormalities in partially lesioned rats which was somewhat of a concern initially. Moreover, we are able to employ just one test apparatus, reducing the time necessary for behavioral shaping of the animals before and after the striatal DA degeneration. The behavioral abnormalities expressed by the neurotoxin treated animals closely resemble motor indices used to diagnose motor deficits in idiopathic PD. Since the PI has returned from sabbatical leave, progress on the research should be much more rapid in the next period of the grant.

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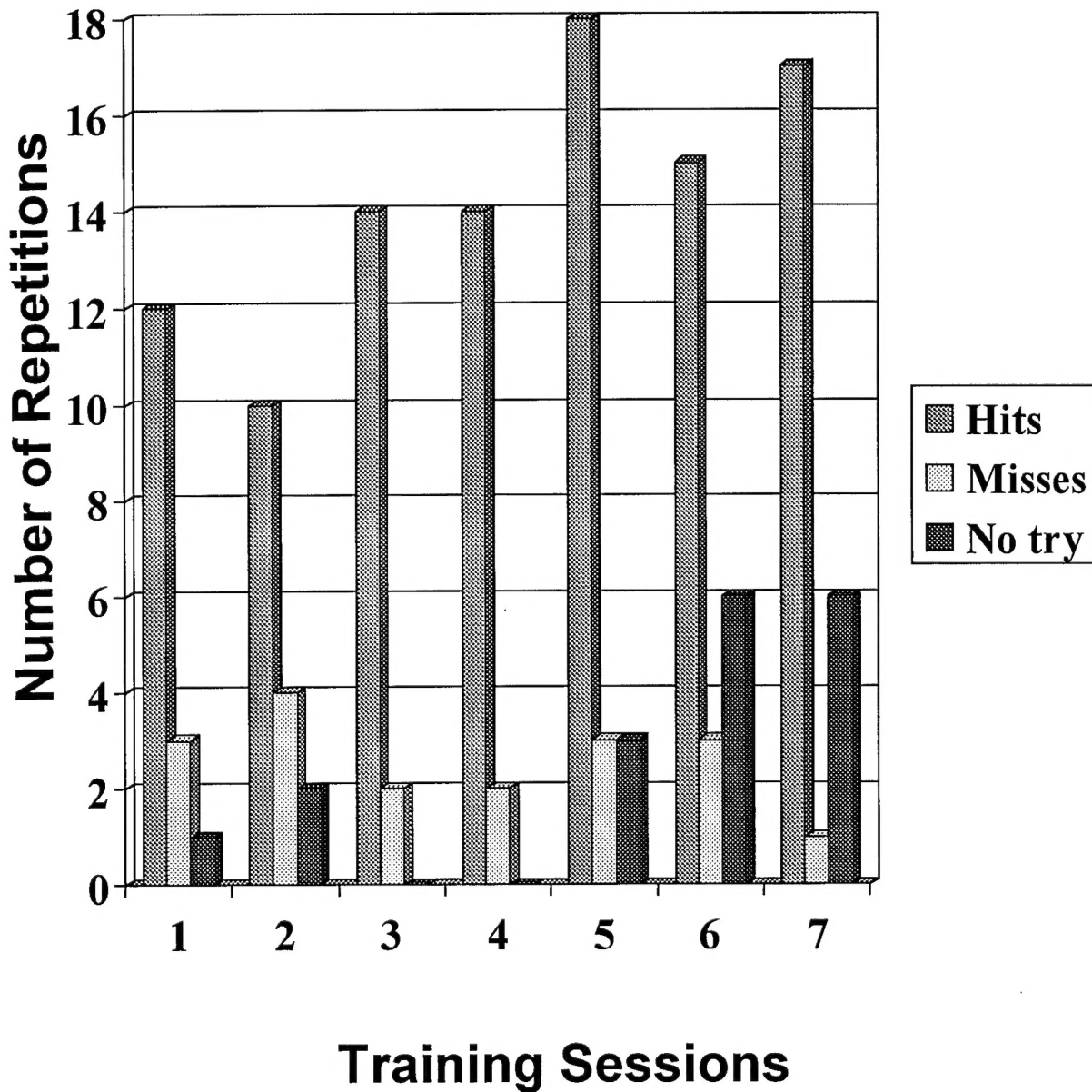
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Appendix:

Graph I. Naïve rat performance on the Montoya staircase apparatus.

Graph II. Neurotoxin-treated rat performance on the Montoya staircase apparatus, before and after bilateral intrastriatal infusion of 6-OHDA.

Graph I. Baseline Performance in Skilled Forepaw Paradigm



Graph II. Skilled Forepaw Performance after 6-OHDA Lesion

